Biosaintifika 12 (1) (2020): 1-9 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Production of Conidia by Entomopathogenic Fungi and Their Pathogenicity Against *Coptotermes* sp.

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Submitted: 11 December 2019. Revised: 11 February 2020. Accepted: 20 March 2020

Abstract. Entomopathogenic fungi have the potential to infect most arthropods including termites which are economically important major insect pests of wood, wood products and building structures. However, the application of this fungus in the field has not shown satisfactory results yet, one of which is constrained in mass production of conidia. The purpose of this study was to evaluate 16 types of biodegradable products and waste as substrates for mass production of conidia using solid state fermentation method and two types of inoculum (solid and liquid inoculum). Toxicity tests were carried out on subterranean termites (*Coptotermes* sp.) based on JIS K 1571, 2010. The parameters observed were the number and dry weight of the conidia produced, conidial viability, nutrient content of the substrate, and percentage of termite mortality. The results showed that rice, sorghum and corn were the best media for the growth of entomopathogenic fungi based on the number of conidia and dry weight of the conidia produced. *Metarhizium* sp. T4.B23 produced the highest number of conidia, 1.12 x 10^{11} conidia/100 g substrate and yielded 180.9 ± 0.623 g dry conidia/kg of rice; followed by *Metarhizium* sp. B2.2 grown on sorghum that resulted in 1.11 x 10^{10} conidia/100 g substrates and 127.8 ± 0.570 g/kg sorghum; and *B. bassiana* produced 8.3 x 10^{9} conidia/100 g substrate and 31.24 ± 0.407 g/kg sorghum. *Metarhizium* sp. B2.2 showed the highest toxicity to termites with 100% mortality that was observed within the second day of testing. The novelty of this research is we found two strains of *Metarhizium* indigenous Indonesia (T4.B23 and B2.2) that isolated from soil in Wangameti, Sumba, East Nusa Tenggara and Mount Gandang Dewata, West Sulawesi, respectively. In this study we not only can produce the high number of conidia from these fungi using cheap media but also it has high toxicity to termites. Furthermore, the conidia of these fungi are very potential to be developed as a biopesticide for termite control, so the use of chemical pesticides can be reduced.

Keywords: *Beauveria*, biopesticide, *Coptotermes*, entomopathogenic fungi, *Metarhizium*

How to Cite: Zulfiana, D., Zulfitri, A., Lestari, A. S., Krishanti, N. P. R. A., & Meisyara, D. (2020). Production of Conidia by Entomopathogenic Fungi and Their Pathogenicity Against *Coptotermes* sp. *Biosaintifika: Journal of Biology & Biology Education*, 12 (1), 1-9

DOI: http://dx.doi.org/10.15294/biosaintifika.v12i1.22435

INTRODUCTION

Coptotermes is a genus of subterranean termite group within family Rhinotermitidae. Genus *Coptotermes'* habitats are widespread in temperate, subtropical and tropical areas (Evans, 2013). Aside for being one of the most efficient lignocellulose decomposers, *C. formosanus* and C. *gestroi* have been recorded as major invasive pests to unprotected wooden constructions and building especially those that contain lignocellulosic materials (Chouvenc, et al., 2015; Tarmadi et al., 2017). In the US alone, economic loss due to termite infestation on man-made structures reached $5 billion annually (Peterson, 2010). There are four different methods to control the insect pests including chemical, mechanical, physical and biological pest-control methods (Indriyanti et al., 2017). However, up to now synthetic chemical termiticide still provides the most efficient and long term solution against termite attack. Types of termite management including pre and post construction, soil treatment and population control are mostly using harmful chemicals classified as Persistent Organic Pollutants (POPs) such as aldrin, coldrane, dieldrin, endrin, etc. (UNEP, 2008). Those chemicals are considered prohibited as pesticide properties due to resistance to degradation thus causing hazardous effects to human and ecosystem. Less toxic chemicals have been used to replace them even though their toxicity still dangerous for non-target insect (Su et al., 2012).

As an alternative to chemical insecticides, biopesticide derived from entomopathogenic fungi have become widely investigated to eliminate local infestations. However, the application method is still being developed because of the nature of termite colonies to avoid infected termite or areas therefore reduce the possibility of epizootic within the colony, especially in subterranean termite (UNEP, 2008; Chouvenc & Su, 2012). Efficiently, the cadaver of infected termite will be immediately cannibalized or buried after being covered with fungistatic materials such as fecal pellets and saliva of termite (Chouvenc & Su, 2012). For that reason, instead of endless work on finding the most virulent fungal against termite, research should be more focused on developing better

formulation and methods for pathogens efficacy in field (Milner, 2003).

A suitable formulation is important to ensure successful utilization and consistent quality of the marketed material throughout the mass-production process (Brownbridge et al., 2001) for example long shelf life and fungal efficacy. In this sense, virulence stability is clearly a desirable trait for a mass-produced biocontrol agent (Ansari & Butt, 2011). An effective incubation conditions in fungal mass production should be applied in order to maintain conidial virulence as explained by Lopez-Perez et al. (2015). Carrier selection is another key factor to maintain and deliver the virulency of main actor of biopesticides when applied on the target. Selecting the right carrier is important since their physico-chemical properties that should not only support the fungal growth for mass production and preserve high survival rate under storage condition but also contribute to the biopesticide formulation efficacy (Kumar et al., 2014).

Entomopathogenic fungi are able to grow on wide range of biodegradable substrates although the most commonly conidial production media selected has been rice grain due to its desired physical characteristics including homogeneity particle size to support a stable production of conidia and structural integrity even after colonization by fungal mycelia (Lopez-Perez et al., 2015). Meanwhile, the use of agroindustrial residues or waste as substrate for production of conidia has been gaining attraction because of the benefits offered such as low cost materials, locally available and derived from non-edible source. Several previous studies have mass produced conidia of entomopathogenic fungi from non grain-based agroproducts as alternatives to grain-based substrate in solid-state fermentation such as rejected raw potato and raw banana (Thakre et al., 2011), rice husk and sugarcane bagasse (Mascarin et.al., 2010), farm yard manure and sugar industry press mud (Prasad & Pal, 2014), jack seed and sawdust (Sahayaraj & Namasivayam, 2008) tea leaf waste, wheat bran, and seed cake of jatropha (Mishra et al., 2016). Even though the use of agricultural waste is considered economical, infection rate of the end product should not be compromised. This study explored a wide rage of biodegradable substrates including grains, cellulosic biomass and agro industrial residues as substrate for spore production of entomopathogenic fungi isolates and also investigated their efficacy against *Coptotermes* sp.

The purpose of this study was to evaluate some types of biodegradable products and waste as substrates for mass production of conidia using solid state fermentation method and two types of inoculum namely solid and liquid inoculum. The result of this study can provide information to the society about the best media that can be used for mass-production of entomopathogenic fungi to be developed as biopesticides, so the use of chemical pesticides can be reduced.

METHODS

Microorganisms and Inoculum Preparation of the Entomopathogenic Fungi

The entomopathogenic fungi isolates used in this study were *Beauveria bassiana* from Bogor Agricultural University Culture Collection (IPBCC), *Metarhizium* T4.B23 isolated from soil in Wangameti, Sumba, East Nusa Tenggara and *Metarhizium* B2.2 isolated from soil of Mount Gandang Dewata, West Sulawesi. The isolates were subcultured on potato dextrose agar (PDA) for 7 days. In this study, two types of inoculums were used: solid inoculums that were grown on PDA media in petri dishes for 7 days and liquid inoculums grown in potato dextrose broth (PDB) media and shaking continuously (120 rpm, 28 ° C) for 3 days.

Preparation of Fungal Growth Substrate

In order to select the best entomopathogenic fungal growth substrate, the study screened 16 types of biodegradable substrates included rice, corn, sorghum, soybean, rice husk, rice bran, sugarcane bagasse, sorghum stalk, rice straw, corn stalk, oil palm empty fruit bunches (OPEFB), sawdust of *Albizia chinensis*, sawdust of *Tectona grandis*, sawdust of *Hevea brasiliensis*, sawdust of bamboo and coconut fiber. Rice grain, corn and shorgums were previously softened by steaming for 1 hour before sterilization process. The substrate were then weighed 100 g and sterilized at 121 °C, 1.5 atm for 15 minutes. After cooled down, each substrates were inoculated with solid and liquid inoculum, respectively. Solid inoculum used was 3 plugs of 6 mm mycelial agar, while 1 ml fungal culture in PDB used as liquid inoculum. Inoculated substrates were then incubated at 25 °C for 14 days.

Determination of Conidia Concentration

Conidial number was counted after incubation period by mixing 1 g of fully colonized substrate into 100 mL of water + tween 80 and vortexed for 5-10 minutes to remove conidia that were attached to the surface of the substrate. Conidia were counted using haemocytometer under microscope at 400 magnification. The number of conidia was determined using the formula by Nuryanti et al. (2012) as follow:

$$J = \frac{t \times d}{0.25 \times n} \times 10^6$$

Note:
J = Total number of conidia in 1 gr of substrate
t = number of conidia in all counted squares
d = dilution (d= 1 (undiluted), d= 10 (diluted 1:10))
0.25 = a constant
n = number of counted squares

Proximate Analysis of Growth Substrate

Proximate composition analysis of the best three growth substrates was conducted in the center for agro-based industry (BBIA, Bogor) following SNI 01-2891-1992. The parameters analyzed were moisture, ash, protein, carbohydrate, lipid and crude fiber.

Harvesting and Viability Test of Conidia

After 14 days, fungus that colonized rice grain were dried in the oven at 30 °C for 3 days before manually shaken through a 60 mesh sieve to harvest the conidia. Collected conidia were then weighed to determine conidia production/kg substrate and stored in -20 °C freezer until further use. To assess viability of the conidia, germination study was conducted monthly during the first 6 month using total plate count (TPC) on PDA.

Bioassay of Dried Conidia against *Coptotermes* sp.

The toxicity of entomopathogenic fungal conidia against soil termites (*Coptotermes* sp.) was carried out using spray and bait method based on modified JIS K 1571, 2010. The both methods were carried out using 50 worker termites and 5 soldier termites. A total of 1 ml of entomopathogenic fungal spore suspension with a concentration of 10^7 was evenly sprayed on the surface of the termite's body in a separate container for each fungal strain and left for 1 minute. Then, the sprayed termites were placed into a 5 mm petri dish that has been lined with thick hard plaster of paris to maintain moisture. Meanwhile, the bait method was performed using paper disc (Whatman 8 mm in diameter) impregnated with 50 µl of entomopathogenic fungal conidia suspension and air dried for 1 minute. After that, the paper disc was put into a 5 mm Ø petri dish which had been coated with plaster of paris and contained 50 worker termites and 5 soldier termites. Each treatment was replicated 3 times and incubated at 25 °C. Number of dead termites was observed every 2 days for 14 days in each treatment. The percentage of termite mortality was calculated by the formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead termite}}{55} \times 100$$

RESULTS AND DISCUSSION

The Number of Entomopathogenic Fungal Conidia in Various Growth Substrate

In this study, 16 types of biodegradable substrates were screened as a medium for mass production of entomopathogenic fungi *B. bassiana*, *Metarhizium* T4.B23 and *Metarhizium* B2.2. Based on the number of conidia per 100 g of substrate, half cooked rice was the best growth medium for entomopathogenic fungi shown by the highest number of conidia produced, followed by sorghum and corn. On rice, the number of *B. bassiana* conidia was ranged from 5.35 x 10^9 to 8.3 x 10^9; *Metarhizium* T4.B23 conidia number were varied between 1.06 x 10^{10} - 1.12 x 10^{11} while *Metarhizium* B2.2 produced 1.06 x 10^{10} - 1.11 x 10^{10} conidia. While on sorghum, conidia counted were between 7.7 x 10^9-7.8 x 10^9; 1.19 x 10^{10} and 8.65 x 10^9 - 8.95 x 10^9, respectively (Table 1). This finding is supported by Bich et al., (2018) that rice is the best medium for the propagation of *M. anisopliae* and *B. bassiana* conidia, while sorghum is the best medium for the production of *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *Verticillium lecanii* (Zimm) Viegas (Sahayaraj & Namasivayam, 2008). Table 1 also shows that the use of two types of inoculums, solid (PDA) and liquid inoculums (PDB) did not significantly affect the number of conidia produced in respective growth substrate.

The number of conidia produced by the three strains of entomopathogenic fungi was also directly proportional to the weight of the conidia per kg of substrate. Fungal strains grown in rice, sorghum and corn produced the highest amount of conidia harvested through the sieving method (Table 2 and Figure 1). The highest conidial yield of *Metarhizium* sp. T4.B23 was produced when using rice as growth medium (180.9 ± 0.623g/Kg rice), this was also as the highest number of conidia compared to other strains grown on the same grain. Meanwhile *Metarhizium* sp. B2.2 and *B. bassiana* reached the maximum yield on sorghum respectively 127.8 ± 0.570 g/ Kg sorghum and 31.24 ± 0.407 g/ Kg sorghum. These results are much higher than those of reported by Me´ndez-Gonza´lez et al. (2017), who obtained conidial yield of only 31.1 g/ kg of rice. Based on conidial yield parameters, rice and sorghum produced almost the same number and weight of conidia, this is probably due to similar nutrient content of both grains especially carbohydrate and protein. However, when economically compared, the abundance of raw materials, and price, rice is a better option than sorghum.

Table 1. The number of conidia of entomopathogenic fungi in 100g growth substrate inoculated with solid and liquid inoculum

Growth substrate	*B. bassiana*		*Metarhizium* sp. T4.B23		*Metarhizium* sp. B2.2	
	PDA	PDB	PDA	PDB	PDA	PDB
Rice	5.35×10^9	8.3×10^9	1.06×10^{10}	1.12×10^{11}	1.06×10^{10}	1.11×10^{10}
Corn	1.5×10^7	2.45×10^7	5.9×10^9	9×10^9	8.5×10^9	2.3×10^9
Sorghum	7.8×10^9	7.7×10^9	1.19×10^{10}	1.19×10^{10}	8.65×10^9	8.95×10^9
Soybean	7.7×10^6	6.15×10^6	4.5×10^3	4.5×10^3	5×10^3	7×10^3
Rice bran	2.85×10^4	2.65×10^4	3.7×10^5	5.5×10^5	7.05×10^5	6×10^5
Rice husk	2.3×10^5	3.35×10^5	5.1×10^4	2.85×10^4	7.5×10^4	5.5×10^4
Bagasse	3.5×10^5	2.5×10^5	9×10^5	3.5×10^5	2.5×10^5	2.5×10^5
Sorghum stalk	8×10^6	1.4×10^6	7×10^6	9×10^6	9×10^6	2×10^6
Rice straw	3.2×10^5	1.5×10^5	7.5×10^5	1×10^5	4×10^5	5.5×10^5
Corn stalk	2.3×10^7	2.1×10^7	3.65×10^7	1.9×10^7	5.8×10^7	1.35×10^7
OPEFB	9×10^4	6.5×10^4	4×10^5	3×10^5	5.5×10^5	3×10^5
Sawdust of *A.chinensis*	2.5×10^5	6×10^5	8×10^5	1.05×10^5	3.5×10^5	2.5×10^5
Sawdust of *T. grandis*	3.5×10^4	6.5×10^4	1.9×10^5	3.5×10^5	4.5×10^5	5×10^5
Sawdust of *H. brasiliensis*	5×10^4	6×10^4	1.8×10^5	8×10^5	2×10^5	6×10^5
Sawdust of bamboo	2×10^6	1.65×10^6	1.55×10^6	8×10^6	3.7×10^6	6.5×10^6
Coconut fiber	4×10^4	4×10^4	3×10^5	3×10^5	3×10^5	2.5×10^5

According to Sahayaraj and Namasivayam (2008), the success of controlling insect pests by microorganisms depends not only on their isolation, characterization and pathogenicity, but also on the mass production of microbial agents in the laboratory. Additionally, for a successful integrated pest management program, the carrier of the biological agents such as entomopathogenic fungi must be easy to obtain and inexpensive such as rice grain. Therefore, as stated by Roshandel et al. (2016) rice is the best substrate to support the growth and development of fungi to get high numbers of conidia of entomopathogenic fungi, while other cereals can also be used as alternatives.

Proximate Analysis of Growth Substrate

Based on the proximate analysis, nutrient content of the three best growth substrates namely rice, sorghum and corn (Figure 2) is listed in Table 3. All tested grains have a high carbohydrate and protein content, which are the main elements needed for fungal growth and conidia production. Tested rice grain contains 9.14% protein and 72.9% carbohydrate, sorghum contains 7.44% protein and 79.1% carbohydrate, while corn contains 10.9% protein and 65% carbohydrate.

Table 2. Average of conidial dried weight harvested from 3 growth substrate

Growth Substrate	Average of conidial dried weight (g/kg substrate)		
	B. bassiana	*Metarhizium* T4.B23	*Metarhizium* B2.2
Rice	30.16 ± 0.236	180.9 ± 0.623	122.84 ± 0.461
Corn	17.84 ± 0.134	39.01 ± 0.091	34.19 ± 0.372
Sorghum	31.24 ± 0.407	167.71 ± 0.580	127.8 ± 0.570

Figure 1. Appereance of harvested conidial grown on rice. *B. bassiana* (left), *Metarhizium* T4.B23 (middle) and *Metarhizium* B2.2 (right).

Figure 2. Entomopathogenic fungal growth on the best three growth substrate

Table 3. Proximate analysis of the three best growth substrates

Growth substrate	Content (%)					
	Water	Ash	Protein (N x 6.25)	Fat	Carbohydrate	Crude fiber
Rice	13.1	0.45	9.14	0.77	72.9	0.63
Corn	13.9	1.99	10.9	4.44	65	3.77
Sorghum	10.6	0.51	7.44	1.22	79.1	1.13

According to FAO (2004), rice provides high carbohydrate but low protein content. A hundred gram of rice contains 79.34 grams of carbohydrates, 0.58 grams of fat and 6.6 grams of protein. Rice is also known to be one of the good mediums for growth of *B. bassiana*, because rice contains a staple carbohydrate. Protein and carbohydrates are needed by fungi for vegetative growth and conidia formation, thus formed conidia will germinate faster and have high virulence if carbohydrate and protein requirements are met (Mishra, et al., 2016).

Nutritional sources can affect growth, sporulation and virulence of the insect pathogenic fungus (Islam et al., 2019). A fairly dominant protein and carbohydrate content in half cooked rice is likely to influence the amount of conidia produced while macro elements such as oxygen, sulfur, and phosphate are the main components of nutrients needed by fungi (Indrayani & Prabowo, 2010). In addition, the surface area of the growth substrate also affects the amount of conidia produced by the fungus. The wider surface area of the substrate, the more conidia will be produced. Substrate that tend to clot will have a narrow surface area,

so that conidia production will be less (Susilawati, 2015).

Viability Test of Entomopathogenic Fungal Conidia

Viability test showed that entomopathogenic fungus conidia were still viable from 1 to 6 months storage in 4 °C refrigerator which verified by germination test on PDA media (Table 4). In this study, after being stored for six months, the three strains of entomopathogenic fungus demonstrated percentage of germination up to 10^5-10^9 CFU/ml despite a decrease in conidial density.

Table 4. Viability test of *B. bassiana*, *Metarhizium* sp. T4.B23 dan *Metarhizium* sp. B2.2 during 6 month storage

Storage time	*B. bassiana* (CFU/ml)	*Metarhizium* sp. T4.B23 (CFU/ml)	*Metarhizium* sp. B2.2 (CFU/ml)
1 month	3.7×10^7	5.4×10^{10}	7.6×10^{10}
2 months	2.1×10^7	1.2×10^9	2.7×10^9
3 months	1.1×10^6	5.1×10^9	2.1×10^9
4 months	11.2×10^5	4.4×10^9	4.8×10^9
5 months	7.2×10^5	4.7×10^9	5.2×10^9
6 months	3.2×10^5	1.7×10^9	3.2×10^9

Figure 3. Germination test of *Metarhizium* sp. B2.2 after 4 months storage (left) and *Metarhizium* sp. T4B23 after 2 months storage (right)

Toxicity of Entomopathogenic Fungal Conidia against Subterranean Termites (*Coptotermes* sp.)

The efficacy of tested entomopathogenic fungus conidia grown on rice media against subterrranean termites was carried out using two methods; spray and bait method for 14 days of treatment. The percentage of termite mortality is shown in Figure 4 where all three strains of entomopathogenic fungus; *B. bassiana*, *Metarhizium* sp. T4.B23 and *Metarhizium* sp. B2.2 demonstrated a high pathogenicity against subterranean termites using either spray or bait method.

On spray method, the conidia of *Metarhizium* sp. B2.2 was effective in killing 100% termite on the 2nd day of testing, while conidia of *Metarhizium* sp. T4.B23 and *B. bassiana* obtained 100% mortality percentage on the 4th and 6th day of testing, respectively. Similar pattern was shown by the results of bait method, *Metarhizium* sp. B2.2 showed the highest toxicity followed by *Metarhizium* sp. T4.B23 and *B. bassiana*. Percentage of termite mortality treated with *Metarhizium* sp. B2.2 reached 100% on the 10th day, while *Metarhizium* sp. T4.B23 killed 100% baited termite on the 12th day testing. Lastly, *B. bassiana* promoted 96.45% termite mortality on the 14th day. Based on this efficacy test, *Metarhizium* was more effective and more toxic against *Coptotermes* sp. compared to *B. bassiana*. This is supported by study from Sileshi et al., (2013) showing that *B. bassiana* (PPRC-56 and 9609) were less effective in controlling *Macrotermes* sp. resulted in percentage of mortality between 25-95% and LT_{50} in 8.8 days compared with *M. anisopliae* isolates (PPRC-2 and MM) killed 60-100% tested termites and LT_{50} value was 7.74 days.

When comparing the effectiveness of the two efficacy methods used, it appears that spray method is more effective than bait method. The fungal conidia kill termites faster, i.e. 100% of termite mortality within 2 days of testing in *Metarhizium* sp. B2.2 treatment. Meanwhile, bait method obtained 100% mortality of termites starting on the 10th day after the treatment. This is due to the characteristics of the entomopathogenic fungus that starting the infection via insect cuticle which is different from the entomopathogenic bacteria that attack through oral toxicity.

Entomopathogenic fungi such as *M. anisopliae* infect their host through the mechanism of attaching conidia to the cuticle of the larvae then germinates and form hyphae to penentrate the insect body. The hyphae will grow deeper into tissues in the body of host by releasing enzymes such as proteases, lipolytics, chitinases and others to penetrate the cuticle. After that, *M. anisopliae* fungal hyphae will attack the

host larvae by absorbing body fluids until the host dies and the body hardens. In addition, *M. anisopliae* produces larvicidal toxins such as destruxin A, B, C, D, E and desmethyl destruxin B, cyclopeptide causing cell paralysis in the target organelle (endoplasmic reticulum, nucleus membrane, and mitochondria). Furthermore, it can cause abnormalities in the physique of the host body such as the middle gut, muscle tissue, hemocyst, and malpighi tubules (Aw & Hue, 2017).

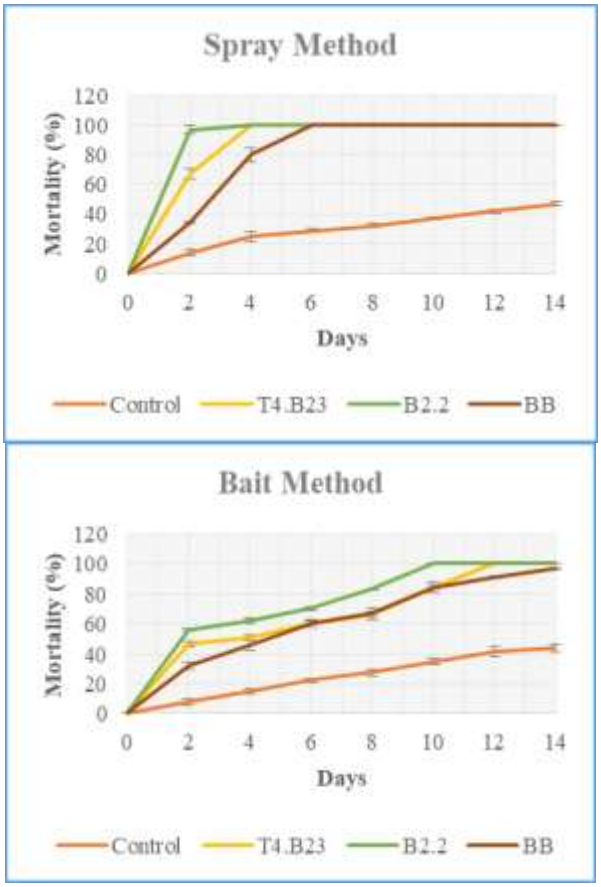

Figure 4. The efficacy of tested entomopathogenic fungus conidia grown on rice media on subterranean termites (a) spraying method (b) bait method

Meanwhile, *B. bassiana* has a slightly different mechanism from *Metarhizium* in infecting its host. According to Keswani et al., (2013), mechanism of infection of the fungus *B. bassiana* starting with hyphae or conidia that penetrate the cuticles in the insect's skin using a variety of enzymes that are able to attack the cuticle of insects such as protease, lipolytic, amylase, and chitinase. Mechanically, the infection can also occur by pressure from the increasing biomass of *B. bassiana* conidium. It starts with the penetration of *B. bassiana* mycelium in the cuticle and apresorium is formed to go through the epidermis and hypodermis. Hyphae that have entered the insect's body will attack the tissue and can multiply in the insect's heamolymph. When the *B. bassiana* fungus is already present in the host's body (target insect), the fungus will produce toxic chemicals such as beauvericin, beauverolide, isorolide, dyes and oxalic acid (Vikhe et al., 2016). Beauvaricin is a poison that causes paralysis, insects will lose coordination in the motion system which causes random movements which then weakened the host and eventually lead to death. These chemicals also have disruptive effects on the respiratory and nerve system, damage the digestive tract and so on (Wahyudi, 2008). Also, according to Wahyudi (2008), after *B. bassiana* succeeds in killing its host, it will be saprophytic fungi. Additionally, *B. bassiana* will release Oosporein substances (antibiotics) that infecting bacteria in the body of the host, so that the growth of *B. bassiana* will spread to all parts of the host body until the mycelium come out from the host body. The fungus growth on the outside of the host body will then form conidia and spread to the surrounding environment to infect new insects target.

The novelty of this research is we found two strains of *Metarhizium* indigenous Indonesia (T4.B23 and B2.2) that isolated from soil in Wangameti, Sumba, East Nusa Tenggara and Mount Gandang Dewata, West Sulawesi. In this study we not only can produce the high number of conidia from these fungi using cheap media but also it has high toxicity to termites. Furthermore, the conidia of these fungi are very potential to be developed as a biopesticide for termite control, so the use of chemical pesticides can be reduced.

CONCLUSION

Rice, sorghum dan corn were the best growth media for entomopathogenic fungi tested (*Metahizium* sp. T4.B23, *Metahizium* sp. B2.2 and *B. Bassiana*) based on the number and weight of conidia produced. *Metahizium* sp. T4.B23 produced the highest number of conidia of all while *Metahizium* sp. B2.2 showed the highest pathogenicity against subterrranean termites compared to other fungal tested.

ACKNOWLEDGEMENT

Authors thank Program Tematik DIPA for funding this research and Sundari from University of Lampung for technical assistant in the laboratory.

REFERENCES

Ansari, M., & Butt, T. (2011), Effects of successive subculturing on stability, virulence, conidial yield, germination and shelf life of entomopathogenic fungi. *Journal of Applied Microbiology*, *110*, 1460-1469.

Aw, K.M.S., & Hue, S.M. (2017). Review: Mode of infection of *Metarhizium* spp. fungus and their potential as biological control agents. *J. Fungi, 3*, 30.

Bich, G. A., Casrtillo, M.L., Villalba, L. L., & Zapata, P. D. (2018). Evaluation of rice by-products, incubation time, and photoperiod for solid state mass multiplication of the biocontrol agents *Beauveria bassiana* and *Metarhizium anisopliae*. *Agronomy Research*, *16*(5), 1921-1930.

Brownbridge, M., Costa, S., & Jarnoski S.T. (2001). Effects of in vitro passage of *Beauveria bassiana* on virulence to *Bemisia argentifolii*. *J. Invertebr Pathol., 77*, 280–283.

Chouvenc, T., & Su, N.Y. (2012). When subterranean termites challenge the rules of fungal epizootics. *PloS one*. *7*(3), 34484.

Chouvenc, T., Helmick, E.E., & Su, N.Y. (2015). Hybridization of two major termite invaders as a consequence of human activity. *PLoS One, 10*, 0120745.

Evans, T.A., Forschler, B.T., & Grace J.K. (2013). Biology of invasive termites: a worldwide review. *Annual Review of Entomology*, *58*, 455–474

FAO. (2004). *Statistical Database of Food Balance Sheet.* Retrieved from *http://www.fao.org. (*Accessed on 3 November 2018).

Indrayani, I.G.A.A., & Prabowo. (2010). Pengaruh komposisi media terhadap produksi konidia jamur entomopatogen *Beauveria bassiana* (Balsamo) Vuillemin. *Buletin Tanaman Tembakau, Serat & Minyak Industri. 2*(2). ISSN: 2085-6717, 88-94.

Indriyanti, D.R., Nuraini, I., & Slamet, M. (2017). The effect of water content of medium containing *Oryctes rhinoceros* larvae on *Metarhizium anisopliae* pathogenicity. *Biosaintifika, 9*(2), 363-369.

Islam, T., Gupta, D.R., Surovy, M.Z., Mahmud, N.U., Mazlan, N., & Islam, T. (2019). Identification and application of a fungal biocontrol agent *Cladosporium cladosporioides* against *Bemisia tabaci. Biotechnology & biotechnological equipment*, *33*(1), 1698–1705.

Test methods for determinating effectiveness of wood preservatives and their performance requirements, [JIS] Japanese Industrial Standard, JIS K 1571-2010; Japanese Standard Association: Tokyo, Japan, 2010.

Keswani, C. Singh, S.P., & Singh, H.B. (2013). *Beauveria bassiana*: Status, mode of action, applications and safety issues. *Biotech Today*, *3*(1), 16-20

Kumar, S., Roy, P.D., Lal, M., Chand, G., & Singh, V. (2014). Mass Multiplication and shelf life of *Trichoderma* species using various agroproducts. *An International Quarterly Journal of Life Sciences*, *9*, 1143-1145.

Lopez-Perez, M, Rodríguez-Gomez, D., & Loera, O. (2015). Production of conidia of *Beauveria bassiana* in solid-state culture: Current status and future perspectives. *Critical reviews in biotechnology*, *35*(3), 334-341.

Mascarin, G.M., Alves, S.B., & Lopes, R.B. (2010). Culture media selection for mass production of *Isaria fumosorosea* and *Isaria farinosa*. *Brazilian Archives of Biology and Technology*, *53*(4), 753-761.

Me´ndez-Gonza´lez, F., Loera-Corral, O., Saucedo-Castan˜eda, G., & Favela-Torres, E. (2017). Current developments in biotechnology and bioengineering: Bioreactors for the production of biological control agents produced by solid-ttate fermentation. (Editor: A. Pandey, C. Larroche, C.R. Soccol, Ed.). Elsevier.

Milner, R.J., (2003). Application of biological control agents in mound building termites (Isoptera: Termitidae) – Experiences with *Metarhizium* in Australia. *Sociobiology*, *41*, 419–428.

Mishra, S., Kumar, P., & Malik, A. (2016), Suitability of agricultural by-products as production medium for spore production by *Beauveria bassiana* HQ917687. *Int. J. Recycl. Org. Waste Agric., 5*(2, 1), 179–184.

Nuryanti, N.S.P., Wibowo L., & Azis A. (2012). Penambahan beberapa jenis bahan nutrisi pada media perbanyakan untuk meningkatkan virulensi *Beauveria Bassiana* terhadap hama walang sangit. *J. HPT Tropika*, *12*(1), 64-70.

Peterson, C. (2010). Considerations of Soil-Applied Insecticides for Termite Control. *Outlooks Pest. Manag.* (21), 89-93.

Prasad, C.S., & Pal, R. (2014). Mass production and economics of entomopathogenic fungus, *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* on agricultural and industrial waste, *Scholars Journal of Agriculture and Veterinary*, *1*(1), pp. 28–32.

Roshandel, S., Askary, H., Hassanlouei, R. T., & Allahyari, H. (2016). The effects of natural substrates on the sporulation and viability of conidia and blastospores of *Metarhizium anisopliae*. *Biocontrol in Plant Protection, 4*(1), 94-103.

Sahayaraj, K., & Namasivayam, S.K.R. (2008). Mass production of entomopathogenic fungi using agricultural products and by products. *African Journal of Biotechnology*, 7. 10.5897/AJB07.778.

Sileshi, A., Sori, W., & Dawd, M. (2013). Laboratory evaluation of entomopathogenic fungi *Metarhizium anisophilae* and *Beauveria bassiana* against termite, Macrotermes (Isoptera: Termitidae). *Asian Journal of Plant Science*, *12*(1), 1-10.

[SNI] Standar Nasional Indonesia, SNI 01-2891-1992. Cara Uji Makanan dan Minuman. Badan Standarisasi Nasional: Jakarta 1992.

Su, N.Y., Lagnaoui, A., Wang, Q., Li, X., & Tan, S. A. (2012). Demonstration Project of Stockholm POPs Convention to Replace Chlordane and Mirex with IPM for Termite Control in China, *Journal of Integrated Pest Management*, *3*(4), D1–D8.

Susilawati. (2015). Sporulasi dan viabilitas konidia cendawan entomopatogen *Beauveria bassiana (Balsamo) Vuillemin* di berbagai media tumbuh [Undergraduate thesis]. Institut Pertanian Bogor.

Tarmadi, D, Yoshimura, T., Tobimatsu, Y., Yamamura, M., Miyamoto, T., & Miyagawa, Y., Umezawa, T. (2017). The effects of various lignocelluloses and lignins on physiological responses of a lower termite, *Coptotermes* f*ormosanus*. *J. Wood Sci. 63*, 464–472.

Thakre, M., Thakur, M., Malik, N., & Ganger, S. (2011). Mass scale cultivation of entomopathogenic fungus *Nomuraea rileyi* using agricultural products and agro wastes. *Journal of Biopesticides*, *4*(2), 176-179.

UNEP/FAO/Global IPM Facility Expert Group on Termite and Biology Management. (2008). Finding Alternatives to Persistent Organic Pollutants (POPs) for Termite Management. United Nations Environment Programme (UNEP)

Vikhe, A. G., Dale, N.S., Umbarkar, R.B., Labade, G.B., Savant, A. R., & Walunj, A.A. (2016). In Vitro and In Vivo Induction, and Characterization of Toxins Isolated from *Beauveria bassiana*. *Int. J. Pure App. Biosci*., *4*(3), 97-103.

Wahyudi, P. (2008). Enkapsulasi propagul jamur entomopatogen *Beauveria bassiana* menggunakan alginat dan pati jagung sebagai produk mikoinsektisida. *Jurnal Ilmu Kefarmasian Indonesia*, 51-56.